WEST Search History

DATE: Monday, March 24, 2003

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ		
L8	L7 and estrogen$	4	L8
L7	mendelsohn-m$.in.	50	L7
L6	L5 and (vascul$ near5 endothel$ near5 cell$)	225	L6
L5	estrogen$ near5 receptor$	3806	L5
L4	L3 and l1	0	L4
L3	barbosa-m$.in.	55	L3
L2	estrogen$ and ((tumor necros$ factor$) near5 receptor$)	313	L2
L1	estrogen$ and (tumor necros$ factor$)	1353	L1

END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 20020115117 A1
TITLE: ERbeta-mediated gene expression

Summary of Invention Paragraph (4):
[0004] Estrogen, a steroid hormone, is involved in growth, differentiation and function of various target tissues in the female and male reproductive systems, including the male prostate gland. Estrogen is also plays certain roles in the central nervous system and the cardiovascular system. Estrogen crosses cell membranes and exerts its effects through binding with high affinity to an estrogen receptor (ER) in the cell nucleus.

Summary of Invention Paragraph (5):
[0005] Historically, the actions of estrogens/antiestrogens were thought to be mediated by the classical ER, now called ER.alpha.. In human and rodent prostates, ER.alpha. is localized in the stromal compartment and basal epithelial cells of the prostate (Prins et al., Endocrinology, 138:1801-1809, 1997; Bonkhoff et al., Am. J. Pathol., 155:641-647, 1999; Wernert et al., Virchows Arch. A. Pathol. Anat. Histopathol, 412:387-391, 1988; Ehara et al., Prostate, 27:304-313, 1995; Kirschenbaum et al., J. Androl, 15:528-533, 1994; Hiramatsu et al., Histopathology, 28:163-168, 1996). Because ER.alpha. is not expressed in the normal glandular epithelium of rat or human prostate (Hartley-Asp et al., Mutat. Res., 143:231-235, 1985; Lau et al., Endocrinology, 139:424-427, 1997; Bonkhoff et al., Am. J. Pathol. 155:641-647, 1999; Ehara et al., Prostate, 27:304-313, 1995; Kirschenbaum et al., J. Androl., 15:528-533, 1994, Hiramatsu et al., Histopathology, 28:163-168, 1996; Rohlff et al., Prostate, 37:51-59, 1998), it has seemed likely that the action of estrogen/antiestrogen on normal prostate epithelial cells (PrECs) is indirect, likely mediated via estrogen-induced stromal factors.

Summary of Invention Paragraph (9):
[0009] In view of such conflicting reports, the roles played by estrogens in the neoplastic transformation of PrEC, and in prostatic carcinoma progression and treatment, have been controversial. Exposure of human or rodent to estrogens induces a proliferative lesion, squamous metaplasia, and in their prostates (Sugimura et al., Hum. Pathol, 19: 133-139, 1988; Yonemura et al., Acta. Anat. (Basel), 153:1-11, 1995; Triche et al., Lab. Invest, 25:596-606, 1971; Levine et al., J. Urol., 146:790-793, 1991), while prolonged treatment of Noble rats with androgen plus estrogen causes a high incidence of prostate adenocarcinoma in the dorsolateral prostates of the treated animals (Noble et al., Cancer Res., 40:3547-3550, 1980; Drago, Anticancer Res., 4:255-256, 1984; Leav et al., Prostate, 15:23-40, 1995; Bosland et al., Carcinogenesis, 16: 1311-1317, 1995). Paradoxically, DES, TAM and other estrogens have been used as treatment regimens for advanced metastatic prostate cancer (Ahmed et al., Int. Urol. Nephrol, 30(2): 159-64, 1998; Bergan et al., Proc. Amer. Soc. Clin Oncol, 14:A637, 1995; Bergan et al., Clinical Cancer Res., 5:2366-2373, 1999; Klotz et al., J. Urol., 161:169-172, 1999; Smith et al., Urology, 52:257-260 1998). In addition to acting as chemical castration agents, both estrogen and antiestrogen are believed to exert direct growth inhibitory effects on prostatic cancer cells via induction of apoptosis or cell cycle arrest (Brehmer et al., J. Urology, 108:890-896, 1972; Hartley-Asp et al., Mutat. Res., 143:231-235, 1985; Schulze et al., Prostate, 16:331-343, 1990; Robertson et al., J. Natl. Cancer Inst., 88:908-917, 1996; Landstrom et al., Int. J. Cancer, 67:573-579, 1996). Details of how estrogens and antiestrogens elicit these responses has remained unclear.

Summary of Invention Paragraph (11):
[0010] The invention is based on the discovery that: (1) normal human prostate

epithelial cells (PreCs) and certain prostate cancer cells express exclusively ER.beta., (2) estrogen/anti-estrogen action in normal and malignant prostate epithelial cells is mediated by ER.beta., and (3) anti-estrogen-induced cell growth inhibition (cell death/apoptosis/cell cycle arrest) in prostate cancer cells is mediated through an ER.beta. signaling mechanism. In addition, there are seven genes in prostatic cancer cells that display ER.beta.-mediated up-regulation in response to the antiestrogen ICI 182,780. Furthermore, ER-.beta. is exclusively expressed in the nuclei of basal cells in normal prostate and down regulation of ER.beta. expression occurs during prostatic carcinogenesis. Specifically, ER-.beta. is not expressed in high-grade dysplasia or in grade 4/5 neoplasms, but is expressed in grade 3 lesions. ER-.beta. is also expressed in metastatic lesions.

Brief Description of Drawings Paragraph (3):
[0019] FIGS. 2A-2D are graphs summarizing data on effects of antiestrogens and estrogens on cell growth of DU145 cells. Cells (5.times.10.sup.3 cells per well) were plated in triplicate wells onto a 24-well plate. After 24 hours for cell attachment, cells were treated for 4 days with antiestrogens [(A) ICI and (B) 4OH-TAM] or estrogens [(C) 17.beta.-estradiol and (D) DES] at various concentrations as indicated. Cells treated with vehicle (absolute ethanol) were used as control. The number of viable cells at the end of 4 days of treatment was determined by the trypan blue exclusion method. Three individual experiments were performed. (Columns, means; bars, S.D.; n=9; *, p<0.001 compared to control (shaded column).

Brief Description of Drawings Paragraph (4):
[0020] FIGS. 3A-3D are graphs summarizing data on effects of antiestrogens and estrogens on cell growth of PC-3 cells. Cell growth assay was described in FIG. 3. (A) ICI. (B) 4OH-TAM. (C) 17.beta.-estradiol. (D) DES. Columns, means; bars, S.D.; n=9; * ,p<0.001; #, p<0.01; +, p<0.05; compared to control (shaded column).

Brief Description of Drawings Paragraph (7):
[0023] FIGS. 6A and 6B are representations of the structures of various phenyl vinyl substituted estrogens (6A) and a Table (6B) showing the effects of various phenyl vinyl substituted estrogens (a type of candidate compound) on cell proliferation in DU-145 cells. %Cell# indicates the percentage of cells surviving after treatment with a candidate compound compared to untreated controls. MW is the molecular weight of the candidate compound.

Brief Description of Drawings Paragraph (8):
[0024] FIGS. 7A and 7B are representations of the amino acid and nucleic acid sequences of human estrogen receptor beta.

Detail Description Paragraph (3):
[0026] Since estrogens are involved in the development and maintenance of the neuronal tissues, identification of compounds that reduce (rather than promote) ER.beta.-mediated apoptosis in neuronal tissues may be desirable, and can be identified using an in vitro screening method of the invention. In some embodiments of the invention designed to identify compounds particularly suitable for neurological use, a test cell of neurological origin is used. Test cells having other origins, e.g., cardiovascular cells or bone progenitor cells, can employed as appropriate, where identification of compounds to treat particular tissues is sought.

Detail Description Paragraph (5):
[0028] The test cell can contain other natural ER subtypes, e.g., ER.alpha., or genetically engineered ER molecules in addition to ER.beta.. In addition, the test cell can contain an estrogen receptor co-regulator (co-activator or co-repressor). However, a test cell containing exclusively ER.beta. (and optionally an estrogen receptor co-regulator) advantageously reduces the probability of "false positives," i.e., candidate compound-dependent gene expression changes that are not ER.beta.-mediated. To promote reliability and consistency in screening assay results, levels of one or more ER subtypes in test cell lines can be assayed periodically. Such assays can be based on ER protein level, mRNA level, or both. ER protein assays and ER mRNA assays can be performed by a person of ordinary skill in the art, using conventional methods and materials.

Detail Description Paragraph (9):
[0032] In addition to being useful for screening synthetic compounds for potential use as pharmaceutical agents, the methods of the invention can be used to test dietary components, e.g., red wine, for the presence of estrogen mimics that trigger

ER.beta.-mediated processes, thereby affecting physiological function positively or negatively. Similarly, the methods of the invention can be used to test environmental pollutants for the presence of estrogen mimics that may pose health risks involving ER.beta.-mediated processes.

Detail Description Paragraph (10):
[0033] Various compounds can be screened using the new methods. The ligand binding cavity of ER.beta. is different from that of ER.alpha. and therefore it will be possible to develop receptor-specific ligands that may form the basis of novel pharmaceuticals with better in vivo efficacy and side effect profile than currently available drugs to target disease cells that express ER-.beta. only. Several broad classes of compounds can be screened. Useful compounds include those that specifically bind to ER.beta. or are predicted to bind to ER.beta.. Compounds that specifically bind to ER.beta. and do not have significant binding to ER.alpha. are also useful. Such compounds are identified using methods known in the art. Other useful compounds are those known to have estrogen or anti-estrogen activities in one or more tissues or cell types (e.g., prostate or nervous system).

Detail Description Paragraph (11):
[0034] Other compounds that can be screened include ER-.beta. agonists such as anti-estrogen therapeutics (tamoxifen, ICI 164,384, ICI 182,780 (see, e.g., Van Den Bemd et al., 1999, Biochem. Biophys. Res. Commun., 261(1):1-5; PMID: 10405313), and diethylstilbestrol), xenocompounds such as environmental pollutants and industrial waste, dental compounds (2',3',4',5'-tetrachlorobiphenyl-ol, bisphenol A, 4-tert-octylphenol, phytoestrogens (compounds present in the plants, mostly as part of our diet), Quercetin, genistein, resveratrol, and natural estrogens and metabolites (e.g., estradiol, and 17 epiestriol). ER-.beta. antagonists can also be used. Examples include: 5,11-cis-diethyl-5,6,11,12-tetrahydro- chrysene-2,8-diol (see, Meyers et al., 1999, J. Med. Chem., 42(13):2456-68 (PMID: 10395487) and Sun et al., 1999, Endocrinology, 140(2):800-4 (PMID: 9927308).

Detail Description Paragraph (31):
[0045] Treatment of DU145 and PC-3 cells with estrogens/antiestrogens

Detail Description Paragraph (32):
[0046] Cells were seeded at a density of 5.times.10.sup.3 per ml into 24-well plates (Falcon, Becton Dickinson Labware, Lincoln Park, N.J.) in a final volume of 1 ml culture medium with 5% charcoal-stripped FBS. Twenty-four hours following seeding, triplicate wells of cells were treated in with 1 .mu.M, 10 .mu.M and 100 .mu.M of estradiol-17.beta. (E.sub.2), diethylstilbestrol (DES), 4'-hydroxytamoxifen (4OH-TAM) or ICI 182,780 (ICI). E.sub.2, DES, and 4OH-TAM were purchased from Sigma Co. (St. Louis, Mo.) and ICI was a gift from Zeneca Pharmaceuticals (Macclesfield, United Kingdom). Estrogens and antiestrogens were dissolved in absolute ethanol (Sigma Co., St. Louis, Mo.) and added to the media daily. Cell cultures not treated with estrogenic compounds received absolute ethanol as a vehicle control. Total additive ethanol concentrations never exceeded 0.2% throughout the culture period. The cells were re-fed with freshly prepared medium every other day. At the end of a 4-day treatment period, cells in each well were trypsinized and cell count determined by direct counting using the Trypan blue exclusion method. All treatment experiments were repeated at least three times to generate statistically relevant data.

Detail Description Paragraph (38):
[0050] Five primary cultures of normal PrECs (N4#6, N3#5, N3#4, N2#3, N2#2) were established from ultrasound-guided peripheral zone biopsies over a period of 18 months. Upon histological examination, the biopsy cores were all judged to contain only normal prostatic tissue with no BPH or cancerous foci contamination. The primary cell cultures were all early passages (second or third), cobblestone in appearance with no visible fibroblast contamination. Semiquantitative RT-PCR analyses (FIG. 1) demonstrated that normal PrEC cultures retained high levels of androgen receptor (AR) mRNA expression, which usually disappeared in late passage normal PrEC primary cultures or in established PrEC. All five cultures of normal PrECs expressed uniform levels of ER.beta. RNA, and transcripts of the estrogen responsive genes, PR and pS2. In contrast, expression of ER.alpha. mRNA was noticeably absent in all five cultures even when high cycle number PCR (>42 cycles) was used to amplify the cDNA. PrEC.TM. (Clonetics Co), a commercially prepared normal PrEC culture, and BPH-1, a SV-40 immortalized prostatic epithelial cell line, expressed both ER.alpha. and ER.beta., but no PR or pS2 transcripts in PrEC.TM. and only minimal level of PR mRNA in BPH-1.

Detail Description Paragraph (43):
Effect of Anti-estrogens and Estrogens on Cell Growth of DU145 and PC-3

Detail Description Paragraph (44):
[0054] Cell growth analyses showed that the growth of DU145 cells, which only expressed ER.beta. mRNA, was adversely affected by the antiestrogens, ICI and 4OH-TAM (FIGS. 2A and 2B). A dose-dependent inhibition of cell numbers was observed in cultures exposed to ICI for 4 days when compared to control cultures treated with vehicle (absolute ethanol). A 40% reduction ($p<0.001$) in the cell numbers was achieved with an ICI dose of 1 .mu.M. A similar growth inhibitory response was observed when DU145 cells were treated with 4OH-TAM. However, cell number reduction achieved with 1 .mu.M of 4OH-TAM was only around 25% ($p<0.001$). In contrast, exposure of DU145 cells to estrogens (E2 and DES) did not affect cell growth in 4-day exposure experiments (FIGS. 2C and 2D).

Detail Description Paragraph (56):
[0062] There are additional reports in the literature of cell lines expressing only ER-.beta. that include genes regulated by this receptor. Examples include the thymidylate synthase gene, survivin gene, telomerase gene, hTERT gene, a subunit of the enzyme telomerase, tumor necrosis factor-alpha (TNF-alpha) and quinone reductase. See, e.g., Nakayama et al., "Tamoxifen and gonadal steroids inhibit colon cancer growth in association with inhibition of thymidylate synthase, survivin and telomerase expression through estrogen receptor beta mediated system," Cancer Lett., 2000, 161(1):63-71 (PMID: 11078914); Routledge et al., "Differential effects of xenoestrogens on coactivator recruitment by estrogen receptor (ER) alpha and Er.beta.," J Biol Chem., 2000, 275(46):35986-93 (PMID: 10964929); Misiti et al., "Induction of hTERT expression and telomerase activity by estrogens in human ovary epithelium cells," Mol. Cell. Biol., 2000 20(11):3764-71 (PMID: 10805720); An et al., "Estradiol repression of tumor necrosis factor-alpha transcription requires estrogen receptor activation function-2 and is enhanced by coactivators," Proc. Natl. Acad. Sci., USA 1999, 96(26):15161-6 (PMID: 10611355); Srivastava et al., "Estrogen decreases TNF gene expression by blocking JNK activity and the resulting production of c-Jun and JunD," J. Clin. Invest., 1999, 104(4):503-13 (PMID: 10449442).

Detail Description Paragraph (63):
[0065] To make an ER.beta.-specific antibody, an immunizing peptide was selected with aid of the computer programs Protean (DNASTAR, Inc., Madison, Wis.) and Peptool (BioTools, Inc., Edmonton, AB, Canada). A peptide sequence in the F domain of the human ER.beta. receptor (amino acids 449-465) was selected, as there is no homology with estrogen receptor alpha (ER.alpha.) at this region (Mosselman et al., FEBS, Lett 392:49-53, 1996; Gustafsson J A, Semin Perinatol, 24:66-69, 2000). The peptide was custom synthesized by Research Genetics (Huntsville, Ala.) with a format of 4-branch Multiple Antigenic Peptide. Each rabbit (male NZW, 5-6 lbs) was first inoculated with 0.5 mg peptide antigen with complete Freund's adjuvant, and then boosted with 0.25 mg peptide plus Incomplete Freund's adjuvant at day 14, day 21, and every two weeks afterward until a satisfactory serum titer was obtained. A direct Enzyme-Linked ImmunoSorbent Assay (ELISA) was used to assess the immune responses to the peptide antigen (Harlow et al., A Laboratory Manual, 139-242, 1988).

Detail Description Paragraph (87):
[0083] Anti-estrogen receptor beta (ER.beta.), rabbit polyclonal antibody GC-17, diluted at 1:6000; anti-estrogen receptor alpha (ER.alpha.), mouse monoclonal antibody NCL-ER-6F11, diluted at 1:50 (Novocastra, Newcastle upon Tyne, UK); anti-androgen receptor (AR), rabbit polyclonal antibody, diluted to 22.7 .mu.g/ml (Upstate Biotechnologies, Lake Placid, N.Y.); anti-Mib5/Ki67, mouse monoclonal antibody, diluted at 1:50 (Immunotech, Westbrook, Me.) and anti-high molecular weight cytokeratin (HMWC), mouse monoclonal antibody 34.beta.E12 diluted at 1:50 (Enzo Diagnostics, Farmingdale, N.Y.). Immunostaining for Prostatic Specific Antigen (PSA) was done with a Nexus Immunostainer (Ventana, Tuscon, Ariz.) using prediluted reagents.

Detail Description Paragraph (133):
Cytotoxicity Studies with Phenyl Vinyl Substituted Estrogens in DU-145 Cells

Detail Description Paragraph (135):
[0121] DU-145 cells were treated for 4 days in media containing 1 mM of each of the

above reagents after pre-seeding for 2 days in 10% FBS. Various concentrations of Resveratrol.RTM. and ICI-182780 were used as positive controls to confirm previous results, i.e., that these compounds lead to a decrease in cell number of 50-70% at 1 mM respectively under the same culture conditions. The percent cell number after treatment was normalized to cells treated with carrier only. FIG. 6A shows the compounds used, and FIG. 6B shows the results of an experiment in which 17.alpha.-substituted (e.g., phenyl vinyl substituted) estrogens were used in the assay. These data show that the various compounds listed in FIG. 6B range in their ability to inhibit cell proliferation. FIG. 6A shows the structural formulas of the 17.alpha.-E (or Z)-(x-phenyl)-vinyl-E.sub.2, and the structural formula for X. The chart in FIG. 6B shows the results for the 25 different compounds in which the R.sub.1, R.sub.2, and R.sub.3 groups of X are varied, and the E or Z form is designated.

Detail Description Paragraph (141):
[0125] Estrogen exposure has been a postulated etiologic factor in the development of testicular neoplasms, and epidemiologic studies have associated prenatal estrogen exposure with an increased risk for germ cell cancer. To date, however, estrogen receptors have not been identified in germ cell neoplasms. The recent identification of the novel estrogen receptor (ER) beta prompted us to investigate if it is present in germ cell malignancies and thus might facilitate the suspected carcinogenic effects of estrogens.

Detail Description Paragraph (142):
[0126] Thirty-two archived surgical specimens from men undergoing radical orchiectomy for testicular cancer were evaluated for the presence of ER.beta.. Five normal specimens were also examined. All specimens were examined by immunohistochemical stains with both the highly specific anti-human estrogen receptor beta (ER.beta.) antibody (as described supra) and with commercially available ER alpha (ER.alpha.) antibody.

Detail Description Paragraph (144):
[0128] These data provide the first demonstration of the expression of ER.beta. in testicular germ cell tumors. These findings now suggest a potential mechanism for estrogen's putative involvement in germ cell neoplasia. The differential qualitative expression seen in embryonal cells (MGCTs with embryonal elements) shows that assay of ER.beta. may be of prognostic significance and be useful for determining the appropriate treatment for certain testicular cancers.

CLAIMS:

8. The method of claim 1, wherein the test cell contains an exogenous estrogen receptor co-regulator.

L2: Entry 143 of 313 File: PGPB Aug 1, 2002

DOCUMENT-IDENTIFIER: US 20020102589 A1
TITLE: Microarrays and methods for evaluating activity of compounds having estrogen-like activity

Abstract Paragraph (1):
In order to detect the effects of environmental hormones, the effect of chemical substances having estrogen-like activity are detected and evaluated

Abstract Paragraph (2):
This invention is characterized in that DNA fragments containing portions or wholes of genes and/or ESTs (Expressed Sequence Tags) whose expression is affected by chemical substances having estrogen-like activity are immobilized on the basal plate of the microarry.

Summary of Invention Paragraph (2):
[0001] This invention relates to a microarray comprising DNA probes immobilized on the basal plate thereof and a method for evaluating activity of compounds having estrogen-like activity that uses this microarray.

Summary of Invention Paragraph (4):
[0002] So far, the so-called environmental hormones are known to be chemical substances such as those that have estrogen-like activity as natural estrogens, 17.beta.-estradiol for example, do. These environmental hormones are endocrine disrupting chemicals towards the human body and have greatly deleterious effects on each of the tissues within the human body. Therefore, precise evaluation of the activity of environmental hormones, together with detection of environmental hormones, in each of the tissues of the human body is desired. Although detection of environmental hormones by sampling each of the tissues of the human body has been possible, detection of the activity of environmental hormones in each of the tissues has not been possible.

Summary of Invention Paragraph (6):
[0004] In this way, microarrays enable quantitatively detecting DNA contained within a sample and monitoring gene expression and the like. However, methods for evaluating the detection and the effect of the above-mentioned estrogen using microarrays are not established.

Summary of Invention Paragraph (8):
[0005] This invention has been made in view of the current state of affairs as mentioned above, and an objective of this invention is to provide microarrays capable of detecting and evaluating the effect of chemical substances having estrogen-like activity and methods for evaluating activity of compounds having estrogen-like activity, for the purpose of detecting the effect of environmental hormones.

Summary of Invention Paragraph (9):
[0006] The microarray of this invention, which has accomplished the above-mentioned objective, is characterized in that DNA fragments containing portions or the wholes of genes and/or ESTs (Expressed Sequence Tags) whose expression is affected by chemical substances having estrogen-like activity are immobilized on the basal plate of the microarray.

Summary of Invention Paragraph (10):
[0007] In this invention, it is preferable that the genes and/or the ESTs are selected from the group consisting of those whose expression level changes in breast

cancer-derived MCF-7 cells in the presence of estrogen.

Summary of Invention Paragraph (12):
[0009] group (1): genes and/or ESTs whose expression level increases owing to chemical substances having estrogen-like activity,

Summary of Invention Paragraph (13):
[0010] group (2): genes and/or ESTs whose expression level decreases owing to chemical substances having estrogen-like activity, and

Summary of Invention Paragraph (16):
[0013] Furthermore, it is preferable that, in the microarray of this invention, genes and/or ESTs constitutively expressing regardless of the presence or absence of chemical substances having estrogen-like activity are further immobilized on the basal plate of the microarray.

Summary of Invention Paragraph (25):
[0022] group (9): genes and/or ESTs functioning in tissues on which estrogen acts,

Summary of Invention Paragraph (30):
[0027] group (12): genes and/or ESTs whose expression level increases owing to estrogen in cells resistant to antagonists of estrogen, and

Summary of Invention Paragraph (31):
[0028] group (13): genes and/or ESTs whose expression level decreases owing to estrogen in cells resistant to antagonists of estrogen.

Summary of Invention Paragraph (33):
[0030] On the other hand, the method of this invention for evaluating activity of compounds having estrogen-like activity comprises utilizing the microarray of any one of claims 1 to 11.

Summary of Invention Paragraph (34):
[0031] Furthermore, this invention relates to a method for evaluating activity of compounds having estrogen-like activity, the method comprising the steps of:

Summary of Invention Paragraph (35):
[0032] (a) preparing a nucleic acid sample from a cell with which an effect of a compound having estrogen-like activity is to be evaluated,

Summary of Invention Paragraph (41):
[0037] The microarrays and the methods of this invention for evaluating activity of compounds having estrogen-like activity are described in detail below.

Summary of Invention Paragraph (42):
[0038] The microarray to which this invention is applied has multiple DNA fragments immobilized on the basal plate of the microarray. Here, DNA fragments contain a portion or the whole of genes and/or ESTs whose expression is affected by chemical substances having estrogen-like activity. Specifically, examples of the genes and ESTs include those indicating responsiveness towards 17.beta.-estradiol in a breast cancer-derived MCF-7 cell line or MCF-7 cell line-derived cell line. That is, in a breast cancer-derived MCF-7 cell line or MCF-7 cell line-derived cell line, it is possible to use DNA fragments containing a portion or the whole of the genes or ESTs whose expression level changes in response to the presence or absence of 17.beta.-estradiol.

Summary of Invention Paragraph (113):
[0109] estrogen receptor {X03635};

Summary of Invention Paragraph (191):
[0187] Furthermore, in this invention, the genes and/or ESTs to be immobilized on the basal plate are not limited to those belonging to the above-mentioned groups (1), (2), and (3), and any genes and/or any ESTs may be used as long as their expression is affected by chemical substances having estrogen-like activity. The term "chemical substances having estrogen-like activity" used herein means a chemical substance that affects female reproductive organs and such similarly to a human sex hormone, estrogen (a general term for hormones that are steroidal chemical substances secreted from ovarian follicles, placenta, and such and that induce the development of female reproductive organs such as follicles and mammary glands or

other organs (such as brain)). Although the functional mechanism of estrogen itself is not yet completely understood, estrogen is thought to affect the expression of other genes by binding to estrogen receptors. Therefore, estrogen is thought to affect tissues such as brain, liver, muscle, bone cells, stomach, and so on where estrogen receptor gene is expressed.

Summary of Invention Paragraph (192):
[0188] Examples of chemical substances having estrogen-like activity include natural or synthesized female hormones such as 17.beta.-estradiol, estrone, estriol, diethylstilbestrol, and such. In addition, examples of chemical substances having estrogen-like activity include plant estrogens such as coumestrol and daidzein. Furthermore, examples of chemical substances having estrogen-like activity include agricultural chemicals such as DDT, aldrin, and heptachlor. Furthermore, examples of chemical substances having estrogen-like activity include industrial raw materials, materials, products, and by-products such as dioxin, PCB, nonyphenol, bisphenol A, phthalic acid esters, and tributyltin compounds.

Summary of Invention Paragraph (194):
[0190] In addition, it is preferable that, in a microarray, genes and/or ESTs constitutively expressing regardless of the presence or absence of chemical substances having estrogen-like activity (hereinafter, referred to as control genes and the like) are immobilized on the basal plates. For the control genes and the like, it is preferable to select several of those that have differences in their expression levels. Genes constitutively expressing regardless of the presence of absence of chemical substances having estrogen-like activity and having differences in their expression levels are, for example,

Summary of Invention Paragraph (204):
[0200] tumor necrosis factor receptor superfamily, member 7 {AA994925}.

Summary of Invention Paragraph (208):
[0204] The expression levels of the genes and/or ESTs indicating responsiveness towards estrogen in the cell to be evaluated can be measured by using a microarray composed as described above. Measuring the expression level of genes and/or ESTs in the cell to be evaluated enables evaluation of the effects of chemical substances having estrogen-like activity, such as environmental hormones, etc.

Summary of Invention Paragraph (213):
[0209] The effect of chemical substances having estrogen-like activity on the cells to be evaluated can be determined using a microarray by detecting the expression level of the above-mentioned genes and/or ESTs in the cells to be evaluated. In other words, using the above-mentioned microarray to measure the expression of genes and ESTs in these cells, the effects of environmental hormones and such may be evaluated.

Summary of Invention Paragraph (225):
[0217] the evaluation value is calculated. Since this evaluation value becomes a relative value when the expression level of genes and ESTs in MCF-7 cells in the presence of 10 nM 17.beta.-estradiol is taken to be 1.00, it will reflect the effect of chemical substances having estrogen-like activity in the cells to be evaluated.

Summary of Invention Paragraph (231):
[0220] Calculation of the evaluation value in this manner, considering the weighting factors for the genes and ESTs, makes the resulting evaluation value clearly reflect the effect of chemical substances having estrogen-like activity in the cells to be evaluated. For the control genes and such, the weighting factor is taken to be zero in the calculation.

Summary of Invention Paragraph (232):
[0221] Here, weighting factor Kn is not set in a restrictive manner according to genes and/or ESTs but rather, it is preferable that this value is changed, considering the current state of the evaluation results, in order to obtain a more accurate evaluation result. Especially, the value of weighting factor Kn is preferably set to various values depending on the tissue from which the cells to be evaluated are derived, and on the compound having estrogen-like activity. Accordingly, this evaluation method enables a highly accurate evaluation tailored to an objective.

Summary of Invention Paragraph (233):

[0222] In addition, by using the cells obtained from human tissues such as mammary glands, uterus, liver, brain, and stomach as evaluation subjects, this method can distinguish the effect of chemical substances having estrogen-like activity on these tissues. That is, this microarray enables evaluation of the effect of chemical substances having estrogen-like activity on the tissues.

Summary of Invention Paragraph (234):
[0223] In addition, this microarray not only allows evaluation of the effect of chemical substances having estrogen-like activity on the tissues as described above, but it can also elucidate the estrogen activity of known or unknown chemical substances. That is, by using this microarray for chemical substances whose effects on cells are unknown, one can evaluate whether or not these substances have estrogen-like activity.

Summary of Invention Paragraph (235):
[0224] Furthermore, this microarray allows distinguishment of whether an activity is enhanced or inhibited when a chemical substance having estrogen-like activity coexists with another chemical substance.

Summary of Invention Paragraph (236):
[0225] The microarray of this invention not only evaluates the effect of chemical substances having estrogen-like activity on cells to be evaluated, as described above, but can also indicate the details of this effect. Especially, in the above-mentioned microarray, the 138 kinds of genes and ESTs of the groups (1), (2), and (3) can be categorized into

Summary of Invention Paragraph (273):
[0262] estrogen receptor 1 {X03635}.

Summary of Invention Paragraph (289):
[0278] By individually evaluating the expression level of genes and/or ESTs categorized into the groups (4) to (8) in this manner, it is possible to distinguish how chemical substances having estrogen-like activity affect the cells to be evaluated. In other words, it is preferable that, in the microarrays, the DNA fragments containing a portion or the whole of genes belonging to the groups (4) to (8) are immobilized. In this case, the use of microarrays allows distinction of the types of effects of chemical substances having estrogen-like activity on the cells to be evaluated.

Summary of Invention Paragraph (291):
[0280] group (9): genes and/or ESTs functioning in tissues on which estrogen acts,

Summary of Invention Paragraph (294):
[0283] Specifically, examples of genes and/or ESTs of the group (9) functioning in tissues on which estrogen acts include

Summary of Invention Paragraph (296):
[0285] estrogen receptor 1 {X03635}.

Summary of Invention Paragraph (307):
[0296] By evaluating the expression level of genes categorized into the groups (9) to (11) in this manner, the tissues on which the chemical substances having estrogen-like activity has an effect can be distinguished in the cells to be evaluated.

Summary of Invention Paragraph (308):
[0297] Furthermore, the 138 kinds of genes and ESTs of the groups (1) (2), and (3) may be categorized according to the responsiveness towards tamoxifen, which is an antagonist of estrogen. That is, these 138 kinds of genes and ESTs may be classified into

Summary of Invention Paragraph (309):
[0298] group (12): genes and/or ESTs whose expression level increases owing to estrogen in tamoxifen-resistant cells, and

Summary of Invention Paragraph (310):
[0299] group (13): genes and/or ESTs whose expression level decreases owing to estrogen in tamoxifen-resistant cells.

Summary of Invention Paragraph (311):
[0300] Specifically, examples genes and/or ESTs of the group (12) whose expression level increases owing to estrogen in tamoxifen-resistant cells include

Summary of Invention Paragraph (314):
[0303] Examples of genes and/or ESTs of the group (13) whose expression level decreases owing to estrogen in tamoxifen-resistant cells include

Summary of Invention Paragraph (319):
[0308] By evaluating the expression levels of genes classified into the groups (12) and (13) in this manner, the effect of chemical substances having estrogen-like activity can be distinguished using tamoxifen-resistant cells as the targets of evaluation.

Summary of Invention Paragraph (320):
[0309] As described in detail above, this invention provides a microarray that can distinguish the effect of chemical substances having estrogen-like activity on cells to be evaluated. Using this microarray, it is possible to evaluate the effect of environmental hormones and such on cells to be evaluated.

Summary of Invention Paragraph (321):
[0310] In addition, according to this invention, measurement of the expression levels of genes and/or ESTs in the cells to be evaluated may provide a method for evaluating activity of compounds having estrogen-like activity. The use of this method for evaluating activity of compounds having estrogen-like activity enables evaluation of the effect of environmental hormones and such on the cells to be evaluated.

Detail Description Paragraph (6):
[0317] The cDNA sample prepared in (1) was added dropwise to a microarray in which DNA fragments containing a portion or the whole of the genes and/or ESTs indicated in row "Gene Name" of Table 1 shown below were immobilized on a slide glass (Nisshinbo). The ten genes of NOs. 3, 53, 54, 79, 98, 116, 127, 128, 141, and 148 in Table 1 are genes constitutively expressing regardless of the presence or absence of estrogen, and the DNA fragments containing a portion or the whole of these genes were immobilized on the basal plate as control genes. In addition, changes in the expression levels of the ten control genes and the 138 kinds of genes and ESTs in the presence of 10 nM 17.beta.-estradiol in MCF-7 cells are shown in row "BDE" of Table 1. Here, negative BDE values indicate decrease in expression levels.

Detail Description Paragraph (11):
[0320] where (n=1 to 138), and the result was 64.09/96.00=0.668. That is, when the expression level of the genes and ESTs in MCF-7 cells in the presence of 10 nM 17.beta.-estradiol was taken to be 1.00, the expression level in the cells evaluated in this example was 0.668. Thus, the microarray that has DNA fragments containing a portion or the whole of the genes and ESTs of Table 1 distinguished the effect of 17.beta.-estradiol. As these results show, the effect of chemical substances having estrogen-like activity, such as environmental hormones, on cells can be evaluated by the above-mentioned microarray.

Detail Description Table CWU (1):
1 No. Gene Name GenBank ID BDE K E (example) BDE' 1 absent in melanoma 1 U83115 -3.1 1.2 -1.1 -2.61 2 aconitase 2, mitochondrial U87939 -2.3 0.5 1.0 2.37 3 actin, beta X00351 -1.0 0.0 1.1 2.61 4 activating transcription factor 3 N39944 4.9 1.0 0.0 0 5 alanyl-tRNA synthetase D32050 3.5 0.5 1.9 4.5 6 aldo-keto reductase family 1, member C4 T73188 3.6 0.5 1.2 2.84 7 alpha-1-antichymotrypsin X00947 2.7 0.5 2.9 6.87 8 amphiregulin M30704 8.2 1.0 5.0 11.85 9 antigen identified by monoclonal antibodies 4F2, TRA1.10, TROP4, and T43 AB018010 5.2 0.5 2.6 6.16 10 argininosuccinate synthetase AI660571 2.9 0.5 2.7 6.4 11 asparagine synthetase L35946 12.1 0.5 4.2 9.95 12 B-factor, properdin X72875 3.2 0.5 1.0 2.37 13 bassoon Y18448 -3.0 0.5 0.0 0 14 cadherin 18 U59325 -3.7 1.0 1.1 2.61 15 calpain, small polypeptide AC002984 -2.8 0.5 -1.4 -3.32 16 carnitine palmitoyltransferase I, liver L39211 -2.6 1.0 1.2 2.84 17 catenin, delta 2 U96136 -3.2 1.0 -1.2 -2.84 18 cathepsin D M63138 2.4 0.5 2.2 5.21 19 CCAAT/enhancer binding protein (C/EBP), beta AA557306 5.1 1.0 2.1 4.98 20 CDC6 homolog AF022109 2.0 1.0 -1.1 -2.61 21 CDP-diacylglycerol-inositol 3-phosphatidyltransferase AF014807 -2.0 0.5 -1.2 -2.84 22 ohromobox homolog 1 AL046741 -2.1 0.5 -1.4 -3.32 23 clusterin X14723 -3.4 0.5 -1.2 -2.84 24 corneodesmosin L20815 2.2 0.5 -1.1 -2.61 25 cyclin AI U66838 3.4 1.0 0.0 0 26 DKFZP566G223 protein AI261366 3.0 0.5 2.6 6.16 27 down-regulated in adenoma L02785

4.0 1.2 -1.1 -2.61 28 early growth response 3 X63741 8.8 1.0 1.9 4.5 29 EGF-containing fibulin-like extracellular matrix protein 1 U03877 -6.7 1.0 -3.7 -8.77 30 enolase 2 X51956 -2.7 0.5 -1.8 -4.27 31 enolase 3 X56832 -3.3 0.5 -1.6 -3.79 32 estrogen receptor 1 X03635 -3.0 1.5 -1.4 -3.32 33 ESTs AA587912 6.3 0.5 1.3 3.08 34 ESTs AA629308 3.2 0.5 1.2 2.84 35 ESTs AI188401 2.2 0.5 -1.2 -2.84 36 ESTs AI332415 2.1 0.5 -1.2 -2.84 37 ESTs AI492976 2.1 0.5 -1.1 -2.61 38 ESTs AI949781 2.3 0.5 1.1 2.61 39 ESTs N35555 11.4 0.5 3.7 8.77 40 ESTs U82984 3.1 0.5 1.3 3.08 41 ESTs AA682502 3.1 0.5 2.0 4.74 42 ESTs AA975298 3.7 0.5 2.0 4.74 43 ESTs AI767533 2.0 0.5 -1.1 -2.61 44 eukaryotic translation initiation factor 3, subunit 9 U78525 -2.2 0.5 -1.2 -2.84 45 exportin AF039022 3.0 0.5 1.8 4.27 46 ferritin, heavy polypeptide 1 AA102267 2.9 0.5 0.0 0 47 FOS-like antigen 2 X16706 5.1 1.0 1.8 4.27 48 FOS-like antigen 2 AI088306 3.1 1.0 1.5 3.55 49 fructose-bisphosphatase 1 M19922 -2.6 0.5 -1.6 -3.79 50 fucosyltransferase 8 Y17977 -3.2 0.5 -1.6 -3.79 51 GATA-binding protein 3 X55122 -2.6 1.0 -1.5 -3.55 52 general transcription factor II, i, pseudogene 1 AI700706 -2.8 1.0 -1.7 -4.03 53 general transcription factor IIH, polypeptide 2 AI239815 -1.1 0.0 1.1 2.61 54 glutamate-cysteine ligase L35546 -1.1 0.0 1.0 2.37 55 glutamic-oxaloacetic transaminase 1 M37400 3.9 0.5 1.5 3.55 56 glutamine-fructose-6-phosphate transaminase 1 M90516 3.2 0.5 1.1 2.61 57 glycyl-tRNA synthetase D30658 3.1 0.5 1.7 4.03 58 granulin AF055008 -2.2 0.5 -1.0 -2.37 59 H3 histone, family 3B Z48950 2.8 1.0 1.4 3.32 60 heat shock 70kD protein 1 M59828 -3.6 1.0 -2.0 -4.74 61 heat shock 70kD protein 5 AI878886 4.9 0.5 1.9 4.5 62 histone deacetylase 6 AJ011972 2.0 1.0 -1.1 -2.61 63 Homo sapiens clone 23783 mRNA sequence AF054996 -2.0 0.5 -1.2 -2.84 64 Homo sapiens DNA from chromosome 19, cosmid F21856 AC004030 -2.6 0.5 -1.1 -2.61 65 HS1 binding protein N28312 2.0 0.5 1.3 3.08 66 Human clone 23855 mRNA, partial cds U79302 -2.1 0.5 1.0 2.37 67 Human insulin-like growth factor binding protein 5 L27560 -12.8 1.0 1.1 2.61 68 Human mRNA for KIAA0018 gene, complets cds D13643 -3.0 0.5 -1.5 -3.55 69 hyaluronan-mediated motility receptor (RHAMM) U29343 3.5 1.5 1.4 3.32 70 Incyte EST AI185199 3.8 0.5 -1.1 -2.61 71 Incyte EST AI880413 3.0 0.5 1.2 2.84 72 Incyte EST X625858 3.0 0.5 1.7 4.03 73 inhibitor of DNA binding 4, dominant negative helix-loop-helix protein AL022726 2.1 0.5 -1.2 -2.84 74 insulin-like growth factor binding protein 5 AA374325 -7.0 1.0 0.0 0 75 insulin-like growth factor binding protein 4 M62403 3.5 1.0 0.0 0 76 interferon stimulated gene (20kD) X89773 4.0 0.5 1.1 2.61 77 isocitrate dehydrogenase 2 (NADP+) X69433 -3.7 0.5 -1.1 -2.61 78 isoleucine-tRNA synthetase D28473 2.0 0.5 2.3 5.45 79 keratin 6B L42611 1.1 0.0 -1.0 -2.37 80 keratin 8 AI273887 2.2 0.5 1.3 3.08 81 KIAA0058 AL036958 -2.1 0.5 -1.3 -3.08 82 KIAA0123 D21064 -2.0 0.5 -1.1 -2.61 83 KIAA0196 D83780 -2.2 0.5 1.0 2.37 84 KIAA0307 AB002305 -3.6 0.5 -1.8 -4.27 85 KIAA0551 AB011123 2.0 0.5 -1.2 -2.84 86 KIAA0587 AB011159 2.4 0.5 1.5 3.55 87 KIAA1051 AB028974 6.4 0.5 1.7 4.03 88 mal X76220 -2.0 0.5 1.0 2.37 89 mannosidase, alpha, class 1A, member 1 X74837 2.1 0.5 1.4 3.32 90 matrix Gla protein AA484893 8.2 0.5 2.3 5.45 91 membrane component, chromosome 1, surface marker 1 X13425 -2.0 0.5 -1.5 -3.55 92 methylene tetrahydrofolate dehydrogenase X16396 3.9 0.5 2.4 5.69 93 microtubule-associated protein 1B L06237 3.9 0.5 2.1 4.98 94 motilin X15393 4.3 0.5 1.1 2.61 95 MYC promoter-binding protein 1 AA482422 -2.6 1.2 -1.5 -3.55 96 neuropeptide Y receptor Y1 M84755 3.2 1.5 1.4 3.32 97 nuclear receptor interacting protein 1 X84373 3.0 1.5 2.3 5.45 98 nucleophosmin AA173870 -1.1 0.0 -1.1 -2.61 99 paired basic amino acid cleaving system 4 D87993 -3.5 0.5 -1.2 -2.84 100 PDZ domain containing 1 AF012281 3.4 0.5 2.5 5.92 101 phorbol-12-myristate-13-acetate-induced protein 1 D90070 4.2 1.2 1.1 2.61 102 phosphoenolpyruvate carboxykinase 2 X92720 7.6 0.5 2.1 4.98 103 phosphoinositide-3-kinase, class 3 Z46973 3.8 0.5 -1.1 -2.61 104 pituitary tumor-transforming 1 AA430241 2.5 1.2 -1.1 -2.61 105 poliovirus receptor X64116 2.0 1.5 1.1 2.61 106 prostate differentiation factor AA216685 5.0 1.0 1.4 3.32 107 protein geranylgeranyltransferase type I, beta subunit AA481712 2.0 0.5 1.0 2.37 108 protein kinase C substrate 80K-H J03075 -2.2 0.5 -1.0 -2.37 109 protein kinase C, delta D10495 -2.9 0.5 -1.4 -3.32 110 protein tyrosine phosphatase, non-receptor type 18 X79568 -2.0 1.0 1.0 2.37 111 quiescin Q6 U97276 -4.0 0.5 -1.1 -2.61 112 ras homolog gene family, member C L25081 -2.1 1.2 -1.6 -3.79 113 reticulocalbin 1, EF-hand calcium binding domain D42073 2.0 0.5 1.7 4.03 114 retinoblastoma-binding protein 8 U72066 3.9 1.2 1.3 3.08 115 Rho GDP dissociation inhibitor alpha X69550 -3.1 0.5 -1.1 -2.61 116 ribosomal protein L35 AI815757 -1.0 0.0 -1.2 -2.84 117 ribosomal protein S6 kinase, 90kD, polypeptide 3 U08316 3.1 1.0 1.1 2.61 118 S100 calcium-binding protein A9 (calgranulin B) X06233 5.1 0.5 0.0 0 119 S100 calcium-binding protein P AI151190 4.7 0.5 1.6 3.79 120 selenium binding protein 1 U29091 -4.4 0.5 -2.1 -4.98 121 semaphorin AI683760 3.6 0.5 1.4 3.32 122 serine hydroxymethyltransferase 2 U23143 2.2 0.5 1.8 4.27 123 SH3-domain binding protein 5 (BTK-associated) AB005047 2.7 0.5 -1.0 -2.37 124 solute carrier family 1, member 4 L14595 5.5 1.0 2.0 4.74 125 solute carrier family 1, member 5 AF105826 2.4 0.5 1.6 3.79 126 solute carrier family 12, member 2 U30246 -4.3 1.0 -1.6 -3.79 127 solute carrier family 29, member 2 AI342303 -1.1 0.0 -1.1 -2.61 128 solute carrier

family 5, member 1 Z80998 -1.0 0.0 1.2 2.84 129 solute carrier family 7, member 5 M80244 5.3 0.5 2.6 6.16 130 sorbitol dehydrogenase U07361 -2.2 0.5 -1.1 -2.61 131 stanniocalcin 2 AB012664 5.5 0.5 1.0 2.37 132 stearoyl-CoA desaturase AA143530 2.0 0.5 -1.1 -2.61 133 synaptogyrin 2 AJ002308 -2.3 1.0 -1.2 -2.84 134 TATA box binding protein (TBP)-associated factor G U21858 2.1 1.0 1.3 3.08 135 tetraspan 1 AF065388 -3.8 0.5 -1.2 -2.84 136 TGFB inducible early growth response AF050110 3.4 1.0 1.2 2.84 137 thrombospondin 3 AI679881 2.0 0.5 -1.1 -2.61 138 transcobalamin I J05068 6.2 0.5 0.0 0 139 trefoil factor 1 X05030 11.7 1.5 4.9 11.61 140 tryptophanyl-tRNA synthetase X62570 5.7 0.5 2.3 5.45 141 tumor necrosis factor receptor superfamily, member 7 AA994925 -1.1 0.0 1.1 2.61 142 tumor protein D52-like 1 U44427 8.1 1.2 2.1 4.98 143 tumor rejection antigen (gp96) 1 X15187 3.3 1.2 1.5 3.55 144 tyrosyl-tRNA synthetase U89436 2.9 0.5 2.1 4.98 145 U5 snRNP-specific protein, 116 kD D21163 -2.0 0.5 1.0 2.37 146 unc-51 (C. elegans)-like kinase 1 AB018265 3.6 0.5 1.1 2.61 147 v-jun avian sarcoma virus 17 oncogene homolog AI598150 3.0 1.2 -1.3 -3.08 148 zinc finger protein 147 D21205 -1.0 0.0 -1.1 -2.61

CLAIMS:

1. A microarray comprising a basal plate and a DNA fragment immobilized on the basal plate, the DNA fragment comprising a portion or the whole of a gene and/or an EST whose expression level is affected by a chemical substance having estrogen-like activity.

2. The microarray of claim 1, wherein the gene and/or the EST is selected from the group consisting of those whose expression level changes in a breast cancer-derived MCF-7 cell in the presence of estrogen.

3. The microarray of claim 1 or 2, wherein the gene and/or the EST belongs to any one of groups (1), (2), and (3) below: group (1): a gene and/or an EST whose expression level increases owing to a chemical substance having estrogen-like activity, group (2): a gene and/or an EST whose expression level decreases owing to a chemical substance having estrogen-like activity, and group (3): a gene and/or an EST whose expression level changes smaller and whose expression level is higher, compared to the groups (1) and (2).

5. The microarray of any one of claims 1 to 4, further comprising a DNA fragment immobilized on the basal plate, the DNA fragment comprising a portion or the whole of a gene and/or an EST constitutively expressing regardless of the presence or absence of a chemical substance having estrogen-like activity.

8. The microarray of claim 1 or 2, wherein the gene and/or the EST belongs to any one of groups (9), (10), and (11) below: group (9): a gene and/or an EST functioning in a tissue on which estrogen acts, group (10): a gene and/or an EST characteristic of an epithelial cell, and group (11): a gene and/or an EST functioning in a nerve cell.

10. The microarray of claim 1 or 2, wherein the gene and/or the EST belongs to group (12) or (13) below: group (12): a gene and/or an EST whose expression level increases owing to estrogen in a cell resistant to an antagonist of estrogen, and group (13): a gene and/or an EST whose expression level decreases owing to estrogen in a cell resistant to an antagonist of estrogen.

12. A method for evaluating activity of a compound having estrogen-like activity, the method comprising utilizing the microarray of any one of claims 1 to 11.

13. A method for evaluating activity of a compound having estrogen-like activity, the method comprising the steps of: (a) preparing a nucleic acid sample from a cell with which an effect of a compound having estrogen-like activity is to be evaluated, (b) contacting the nucleic acid sample with the microarray of any one of claims 1 to 11, (c) detecting a nucleic acid hybridizing with the microarray, and (d) comparing a result of the detecting of (c) to a result detected using a nucleic acid sample prepared from a control cell.

L2: Entry 299 of 313 File: USPT Dec 8, 1998

DOCUMENT-IDENTIFIER: US 5846720 A
TITLE: Methods of determining chemicals that modulate expression of genes associated with cardiovascular disease

Brief Summary Text (47):
The best examples of promoters subject to pharmaceutical intervention are provided by those of the steroid responsive genes. Nolvadex (Tamoxifen), for example, regulates the expression of estrogen responsive genes in the control of breast cancer, while Eulexin (flutamide) demonstrates dramatic palliative efficacy in the treatment of prostatic carcinoma by blocking the action of the testosterone receptor. Similarly, steroid receptor agonists are used as anti-inflammatory agents, contraceptives, etc. It is now becoming increasingly apparent that a number of drugs whose mechanism was previously unknown, also act by specifically modulating the expression of various target genes. For example, the immunosuppressive agents cyclosporin A and FK506, both inhibit the transcription of several genes involved in T cell activation, in particular interleukin 2 (64, 65). The cellular receptors for these compounds are the proteins termed cyclophilins, which may act, at least in part, by inhibiting the Ca.sup.2+ /calmodulin-dependent protein phosphatase calcineurin. Altered phosphatase activity presumably modulates gene expression by altering the phosphorylation state of specific transcription factors, or transcription factor activity modulators. The anti-inflammatory action of aspirin has also recently been shown to be at least partly due to altered levels of gene expression.

Detailed Description Text (44):
In the methods described above the cardiovascular disease may be atherosclerosis or restenosis. The protein of interest may be involved in lipid transport or cellular uptake e.g. apolipoprotein (a, AI, AII, AIV, B, CI, CII, CIII or E), low density lipoprotein receptor (LDL-R), cholesterol ester transfer protein, hepatic TG lipase, lipoprotein lipase, high density lipoprotein receptor p110, LDL receptor like protein, ARP1, LDL-R protein kinase, apolipoprotein E receptor or oncostatin M. The protein of interest may be involved in the uptake of modified lipoproteins e.g. LDL-R, scavenger receptor, advanced glycosylated end-product receptor or macrophage FC receptor. The protein of interest may be involved in lipid metabolism e.g. AMP-activated protein kinase, AMP-activated protein kinase kinase, acetyl CoA cholesterol ester transferase, lecithin-cholesterol ester transferase, cholesterol 7.alpha.-hydroxylase, hormone sensitive-lipase/cholesterol ester hydroxylase or HMG CoA reductase. The protein of interest may be involved in lipid oxidation e.g. 15-lipoxygenase, IL-4, IL-4 receptor, superoxide dismutase or 12 lipoxygenase. The protein of interest may be involved in smooth muscle cell growth such as platelet derived growth factor (PDGF-A), PDGF-B, PDGF-.alpha. receptor, PDGF-.beta. receptor, heparin-binding EGF-like growth factor, basic fibroblast growth factor (bFGF), aFGF, FGF receptor, IL-1, IL-1 receptor p80, IL-1 receptor protein kinase, interferon gamma, TGF-.beta.1, TGF-.beta.2, TGF-.beta.3, TGF receptor, tumor necrosis factor-.alpha. (TNF-.alpha.), TNF-.alpha. receptor, .alpha.-thrombin, .alpha.-thrombin receptor, 9-hydroxyoctadeca-10,12-dienoic acid (9-HODE) receptor, insulin-like growth factor, platelet factor-4, TGF-.alpha., thromboxane A.sub.2 receptor, 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) receptor, 13-hydoxyoctadeca-9,11-dienoic acid (13-HODE) receptor, IL-6, IL-6 receptor or EGF receptor. The protein of interest may be an endothelial cell growth factor or receptor (EGF) such as vascular EGF, VEGF receptor, bFGF, aFGF, FGF receptor or platelet-derived endothelial cell growth factor. The protein of interest may be associated with macrophage growth and chemotaxis e.g. CSF-1, CSF-1 receptor, monocyte chemoattractant protein-1 (MCP-1) or MCP-1 receptor. Lastly the protein of interest associated with atherosclerosis may be associated with endothelial cell

adhesion such as VCAM-1, VLA-4 .alpha..sub.4 subunit, VLA-4 .beta..sub.1 subunit, ELAM-1, ICAM-1, LFA-1 .alpha..sub.L subunit, LFA-1 .beta..sub.2 subunit, GMP-140 (PADGEM), neuropeptide Y, VLA-4 .alpha..sub.1 subunit, vitronectin receptor or 13-hydoxyoctadeca-9,11-dienoic acid (13-HODE) receptor. The protein of interest associated with the treament of cardiovascular disease or atherosclerosis may be PEPCK.

Detailed Description Text (210):
HepG2 hepatocarcinoma cells have been previously shown to express apolipoprotein AI (71, 86). Hep G2 cells are transfected with 10 .mu.g of linear pApoAI-106 per 10.sup.6 cells using electroporation. Neomycin resistant colonies are isolated and analyzed as outlined above. Factors known to modulate apolipoprotein AI transcription (estrogen, testosterone, cholesterol, thyroid hormone (54) are tested and the best clones selected for use in the high throughput screen.

Other Reference Publication (45):
Pons, M. et al., (1990) "A New Cellular Model of Response to Estrogens: A Bioluminescent Test to Characterize (Anti)Estrogen Molecules," BioTechniques 9(4): 450-459.